

Original Article

INFLUENCE OF CADMIUM ON ANTIOXIDATIVE DEFENCE SYSTEM, PHOTOSYNTHESIS, LEVEL OF OSMOLYTES AND IONS UPTAKE IN *BRASSICA JUNCEA*

DHRITI KAPOOR¹, AMANDEEP RATTAN¹, SATWINDERJEET KAUR¹, RENU BHARDWAJ¹

¹Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar-Punjab (India) 143005
Email: dhriti405@gmail.com

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ABSTRACT

Objective: In the present study various physiological and biochemical aspects of *Brassica juncea* were studied under cadmium (Cd) stress conditions.

Methods: Plants of *Brassica juncea* were subjected to different concentrations of Cd (0, 0.2, 0.4 and 0.6 mmol) metal. After 30 d of cadmium exposure it was found that the level of antioxidants, osmolytes, photosynthetic parameters, ions and total sugars were altered. To investigate the effects of metal in *Brassica juncea* plants, level of ascorbic acid, tocopherol, glutathione, ferric ion reducing assay, molybdate ion reduction assay, total osmolytes content, anthocyanins, xanthophylls, transpiration rate, stomatal conductance, water use efficiency, uptake of sodium and potassium ions, level of carbon, hydrogen, nitrogen, sulfur and total sugar content was detected.

Results: Results from this study revealed the increase in antioxidant potential of *Brassica juncea* plants under cadmium metal stress. Photosynthetic parameters and uptake of sodium and potassium ions were affected negatively due to metal exposure and level of sugars and osmolytes were found to rise in the presence of cadmium stress.

Conclusion: Findings of present study suggested that treatment of Cd activated a range of defence strategies in *Brassica juncea* plants.

Keywords: Cd Stress, Osmoprotectant, Antioxidative defence system, Photosynthesis

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INTRODUCTION

Heavy metal (HM) toxicity is creating trouble for evolutionary, nutritional, ecological, and environmental reasons as these metals are major environmental pollutants [1]. Metal toxicity varies with plant species, specific metal, concentration, chemical form and pH of soil composition in plants [2]. Accumulation of higher doses of metal causes toxicity in different ways in plants. Heavy metals may be divided into two groups: redox active (Cu, Cr, Fe, Co) and redox inactive (Cd, Ni, Al, Zn, etc.) metals. The redox active metals are directly involved in the redox reactions of the cells and trigger the formation of $O_2^{\cdot-}$, H_2O_2 and $\cdot OH$ subsequently via the Haber-Weiss and Fenton reaction [3]. Exposure of plants to redox inactive metals results in oxidative stress through indirect involvement in the mechanisms like disruption of the electron transport chain, interaction with the antioxidant defence system and induction of lipid peroxidation. The most prevalent visual symptoms of HM toxicity is retardation in plant growth [4] including leaf chlorosis, necrosis, turgor loss, reduction in the rate of seed germination and a crippled photosynthetic apparatus, along with progressing senescence processes and finally plant death [5]. Furthermore, HMs affect homeostatic events like water uptake, transport, transpiration and nutrient metabolism [6] and also interfere with the uptake of Mg, K, Ca and P [7]. Elevated levels of HMs usually inhibit photosynthesis due to their direct influence on the photosynthetic apparatus, including thylakoids. HM toxicity triggers the accumulation of excess reactive oxygen species (ROS) inside the cell. Production of ROS depends upon the particular HM element. Cd is a redox-inactive HM, which produces ROS indirectly by inactivation of enzyme and also by inducing the expression of lipoxygenase (LOX) in plant tissues and consequently leads to oxidation of polyunsaturated fatty acids [8].

Various stress protective proteins, compatible solutes and antioxidants are possessed by plants in response to various stress conditions. They protect the plants by increasing tolerance or avoiding the toxicity of metals [9]. *Brassica juncea* belongs to family Cruciferae (Brassicaceae). It is an oilseed crop, mainly grown as a food crop and also used for its medicinal purposes. It contains

antioxidants like carotenes, flavonoids, lutein, indoles, and zeaxanthin [10], which helps in the activation of cellular defence system and biological system against oxidative damage. As *Brassica juncea* plants are hyper accumulator for heavy metals, thus present investigation was done to observe the effects of Cd metal on the level of antioxidants, free radical scavenging capacity, total osmolytes, photosynthetic pigments, gaseous exchange parameters, elemental analysis and sugars in 30 d old *Brassica juncea* plants.

MATERIALS AND METHODS

For experimentation, certified and disease free seeds of *Brassica juncea* were procured from Punjab Agricultural University, Ludhiana (Punjab).

Chemicals and reagents

$CdCl_2$ (Sigma-Aldrich) is used for experimentation.

Antioxidants

Ascorbic acid content was determined by following the method of Roe and Kuether [11]. Tocopherol content was estimated by the method proposed by Martinek [12].

Glutathione content was analyzed by the method given by Sedlak and Lindsay [13]

Radical scavenging assays

Reducing power assay (FRAP) was performed by the method given by Oyaizu [14] and molybdate ion reduction assay was estimated by Prieto *et al.* [15].

Total osmolyte content was analyzed by using vapor pressure osmometer (VPO) (Vapro 5600).

Photosynthetic pigments

Total anthocyanin content was performed by the method of Macinelli [16].

Xanthophylls content was analyzed by Lawrence [17].

Gaseous exchange of plants like transpiration rate, stomatal conductance and water use efficiency were measured with the help of (IRGA) infra-red gas analyzer (Li-COR 6400). Sodium and potassium ion content was measured by flame emission photometer (systronics 128). Ions content was analyzed by method given by Allen *et al.* [18, 19]. The percentage of carbon, hydrogen, nitrogen and sulphur were determined with the help of CHNS analyzer (Elementar Vario ELIII). Total sugars were quantitatively detected by the method given by Scott and Melvin [20]. Sugar content of 30 d old plants were measured by Metrohm Ion Chromatography (Orion-960).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) for scrutinizing the effect of Cd metal on various experiments and expressed as the mean±standard error of three replicates. The *Tukey post hoc test* ($p \leq 0.05$) was applied for the comparisons against control values using assistat version 7.7 beta.

Table 1: Effect of Cd on antioxidants and antioxidant assays in 30 d old *B. juncea* plants

Treatments	Ascorbic acid (mg/g FW)	Tocopherol (mg/g FW)	Glutathione (mg/g FW)	FRAP (%)	Molybdate ion (%)	Total Osmolytes (μ mol/g FW)
0.0 mmol	6.61±0.43 c	3.96±0.55 ab	7.48±0.61 ab	46.45±3.63 c	68.66±3.46 b	163.83±5.38 b
0.2 mmol	8.96±0.92 bc	5.08±0.12 a	7.79±0.33 a	49.19±3.93 bc	75.56±2.23 ab	179.17±5.42 ab
0.4 mmol	11.63±0.84 ab	5.29±0.23 a	8.16±0.55 a	67.11±2.84 a	73.34±1.91 ab	191.63±2.07 a
0.6 mmol	12.98±1.02 a	4.22±0.28 b	8.72±0.33 b	63.15±2.49 ab	83.29±2.32 a	193.23±2.47 a

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mmol) are significantly different (The *Tukey post hoc test*, $p \leq 0.05$) and signify the effect of Cd metal on various antioxidants and antioxidant assays.

It was observed that continuous rise in anthocyanin content from 7.45 to 9.65 mg/g FW occur. A similar trend was noticed in the case of xanthophylls, where 0.4 mmol Cd treatment showed the highest xanthophylls level (9.92 mg/g DW) (table 2).

In the present study, transpiration rate decreased with the Cd toxicity from 1.97 to 1.11m mol H₂O m⁻²s⁻¹ In 30 d old plants; results

RESULTS

In the present study, 1.96 folds increase in the level of ascorbic acid was recorded in 0.6 mmol Cd-treated seedlings (12.98 mg/g FW) in comparison to control (6.61 mg/g FW). Increase in vit-E level was found from 3.96 to 5.29 mg/g FW i.e., from control to 0.4 mmol Cd treatment. Further GSH content got enhanced from 7.48 to 8.72 mg/g FW in Cd-stressed plants (table 1).

Inhibition of molybdate ion was found maximum in 0.6 mmol Cd-treated plants (83.29%) with respect to control (68.66%). Increase in the inhibition of FRAP ion was noted from control (46.45%) to 0.2 mmol Cd (49.19%). Maximum scavenging of FRAP was reported in 0.4 mmol Cd (67.11%) (table 1).

It was found that total osmolyte content was enhanced with the metal treatment. It was recorded maximum in 0.6 mmol Cd treatment (193.23m mol/Kg), followed by 0.4 mmol Cd (191.63m mol/Kg) and 0.2 mmol Cd (179.17m mol/Kg) treatments (table 1).

revealed a decline in stomatal conductance from control (0.29 mol m⁻²s⁻¹) to 0.6 mmol Cd-stressed plants (0.19 mol m⁻²s⁻¹). With increasing doses of Cd, water use efficiency (WUE) was found to decrease from control to 0.6 mmol Cd-treated plants. Its value decreased from 0.2 mmol (3.31 mgCO₂/gH₂O) to 0.6 mmol Cd (3.02 mgCO₂/gH₂O) treated plants (table 2).

Table 2: Effect of Cd on photosynthetic system in 30 d old *B. juncea* plants

Treatments	Anthocyanin (mg/g FW)	Xanthophyll (mg/g DW)	Transpiration rate (m mol H ₂ O m ⁻² s ⁻¹)	Stomatal conductance (mol m ⁻² s ⁻¹)	H ₂ O use efficiency (mg CO ₂ /gH ₂ O)
0.0 mmol	7.45±0.49 b	3.99±0.49 c	1.29±0.002 a	0.29±0.005 a	4.2±0.18 b
0.2 mmol	8.27±0.53 ab	6.91±0.47 b	1.19±0.03 bc	0.24±0.005 ab	3.31±0.42 a
0.4 mmol	8.96±0.42 ab	9.92±0.53 a	1.27±0.03 ab	0.23±0.02 ab	3.43±0.15 a
0.6 mmol	9.65±0.25 a	5.52±0.30 bc	1.11±0.002 c	0.19±0.006 b	3.02±0.26 a

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mmol) are significantly different (The *Tukey post hoc test*, $p \leq 0.05$) and signify the effect of Cd metal on the photosynthetic system.

A steep decline was observed in the ions content due to Cd phytotoxicity from 7.63 to 6.87 ppm and from 7.92 to 6.77 ppm respectively (table 3). C content was found to increase from 32.22 to 38.66%. Similarly, H content was observed maximum in plants subjected to 0.6 mmol Cd stress (5.84%), which was found to

decrease with 0.2 mmol Cd treatment (5.78%). A sharp increase was recorded in S content. The maximum value was seen in the plants exposed to 0.6 mmol Cd toxicity (0.35%). N content sharply declined with 0.4 mmol Cd treatment (1.13%), which were almost 4.07 folds less than the value of control (4.6%) (table 3).

Table 3: Effect of Cd on Ion content in 30 d old *B. juncea* plants

Treatments	Sodium ion (ppm)	Potassium ion (ppm)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)	Total sugar (μ mol/g FW)
0.0 mmol	9.85±0.54 a	9.58±0.22 a	32.22±2.5 b	4.90±0.15 b	4.6±0.1 a	0.12±0.01 b	17.66±1.27 b
0.2 mmol	7.63±0.37b	7.92±1.04 ab	37.05±1 a	5.78±0.8 a	4.19±0.5 ab	0.29±0.01 ab	18.83±1.08 ab
0.4 mmol	7.55±0.44 b	7.32±0.41 ab	38.24±0.5 a	5.62±0.3 ab	1.13±0.05 c	0.35±0.001 a	20.77±0.70 a
0.6 mmol	6.87±0.31 b	6.77±0.22 b	38.66±1.5 a	5.84±0.59 a	3.38±0.3 bc	0.27±0.05 ab	21.17±1.09 a

Data presented in mean±SE. Different letters (a, b, c and d) within various concentrations of Cd (0, 0.2, 0.4 and 0.6 mmol) are significantly different (The *Tukey post hoc test*, $p \leq 0.05$) and signify the effect of Cd metal on various ions and sugar content.

With increasing metal concentration, sugar content was also found to enhance from 0.2 mmol Cd to 0.6 mmol Cd concentration, i.e., from 18.83 to 21.17 μ mol/g FW as compared to control plants (17.66 μ mol/g FW) (table 3). 30 d old plants of *B. juncea* resulted in the accumulation of sorbitol, mannitol, glucose, fructose and cellobiose. Further, in 0.2 mmol Cd stress

increase in the amount of mannitol from 0.589 to 0.734 ppm, glucose from 3.184 to 10.09 ppm and fructose from 0.776 to 8.287 ppm was recorded. Additional peak of sugar namely sucrose was expressed in 0.4 mmol Cd-treated plants. Two additional sugars, arabinose, and xylose were noticed at the highest metal stress of 0.6 mmol Cd (fig. 1, table 4).

Table 4: Concentrations of sugars in 30 d old *Brassica juncea* plants treated with Cd stress

S. No.	Sugars	Concentrations (ppm)			
		Control	0.2 mmol Cd	0.4 mmol Cd	0.6 mmol Cd
1.	Sorbitol	0.054	–	0.233	–
2.	Mannitol	0.580	0.734	0.151	1.230
3.	Glucose	3.184	10.090	2.615	–
4.	Fructose	0.776	8.287	0.581	–
5.	Cellobiose	0.001	–	0.002	0.611
6.	Sucrose	1.561	–	0.040	1.272
7.	Arabinose	–	–	–	8.563
8.	Xylose	–	–	–	3.326

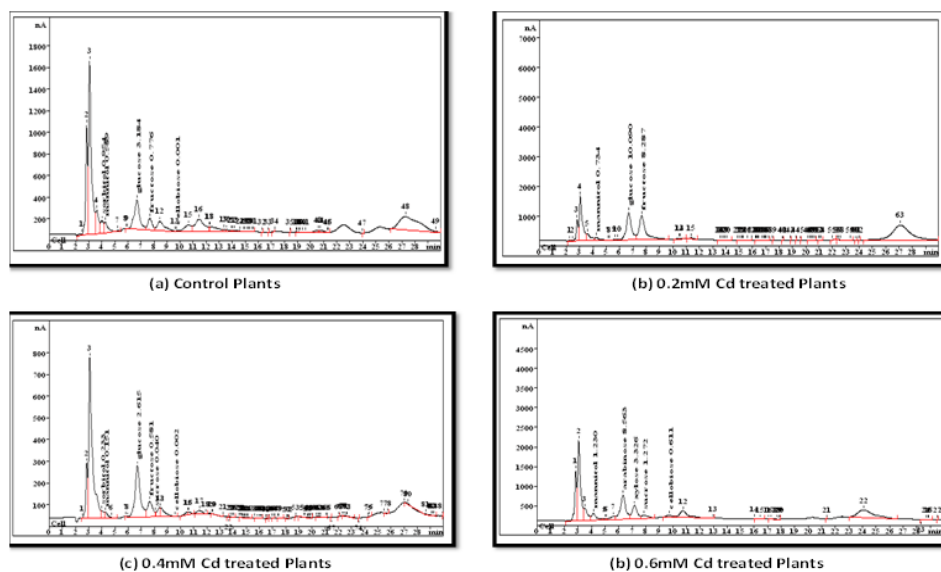


Fig. 1: Qualitative analysis of sugars by Ion chromatography in 30-days old *Brassica juncea* plants treated with Cd

DISCUSSION

In the present study, antioxidant potential of *B. juncea* plants was found to improve with increasing Cd metal stress. Improved antioxidant system acts as a key player in the mitigation of oxidative stress produced by ROS [21]. The observations of the present study were supported by the findings of Choudhary *et al.* [22], where the level of antioxidants like ascorbic acid and GSH were found to enhance in *Raphanus sativus* seedlings exposed to Cr stress. The report suggested that level of GSH increased under Cu and Cd stress in *Cleome gynandra* [23]. Results revealed that level of osmolytes was enhanced in *B. juncea* plants subjected to Cd stress. Osmolytes provide protection against stress; they act as antioxidant by scavenging ROS and stabilizing the membranes [24]. Under stress conditions, Δ 1pyrroline-5-carboxylate synthase enzyme gets stimulated, which further cause an increase in proline content. GB is also accumulated more during the stress conditions as it is formed by choline and GB substrates. Two-step oxidation of choline is stimulated via the toxic intermediate betaine aldehyde and these reactions are catalyzed by choline monooxygenase (CMO) and NAD⁺-dependent betaine aldehyde dehydrogenase (BADH), which is activated under stress conditions [25]. These results are in coherence with the observations of Choudhary *et al.* [22], where Cr toxicity caused a rise in the level of GB.

Present investigation showed the rise in the level of photosynthetic pigments with increasing Cd doses. This is due to the activation of

antioxidative enzyme namely glutathione-S-transferase that causes biosynthesis of anthocyanin pigment [26], acts as a precursor for the synthesis of abscisic acid (ABA), which is also involved in the protection of photosynthesis against oxidative stress [27] and thus also acts as an antioxidant. The observations of present work are in coherence with the observations of Amiri *et al.* [28]. Whereas gaseous exchange parameters such as transpiration rate, stomatal conductance and water use efficiency were found to decline with the Cd treatment. Regarding the toxicity of Cd metal to PSII activities in plants, it has been investigated that Cd binds in the sites of both acceptor and donor sides of PSII [29]. On the donor side, the presence of Cd²⁺ exchanges the Ca²⁺ cofactor in the Ca/Mn cluster that constitutes the oxygen-evolving center with high affinity in a slow reaction that causes reduction of photosynthetic oxygen evolution [30]. Results were supported by the observations of Stancheva *et al.* [31], where water use efficiency (WUE) was decreased in *Ocimum basilicum* and inhibition of gas exchange and transpiration rates were observed in *Origanum vulgare* plants exposed to heavy metal stress.

The level of ions was also declined with Cd toxicity in *B. juncea* plants. Metal treatment negatively affects the uptake and transport of mineral nutrients in the plants [32]. A decline in the level of these ions often indicates their efflux across a plasma membrane and excess dose of metal damage them by inducing the lipid peroxidation. Further, this damage leads to loss of membrane

selectivity and increase in permeability [33]. Results of the present study were supported by Bouazizi et al. [34] where the level of K⁺ was negatively affected in *Phaseolus vulgaris* plants with enhancing Cu concentration. In *Cucumis sativus* plant, Cu toxicity led to a reduction in Na⁺ and K⁺ content [35]. Percentage of carbon, hydrogen and sulphur was found to enhance with increasing Cd doses. The high elemental content in the shoots of *B. juncea* might be related to metal tolerance in accumulators/hyperaccumulators. The level of elements was raised by the enhanced metal concentrations in plant shoots, suggesting that *B. juncea* might have a specific physiological need for them if exposed to potential metal toxicity [36].

In the present study, the level of sugars was noticed to enhance in *B. juncea* plants. Reactive oxygen species react with sugars and oxidize them to release formic acid, which is the main breakdown product. Mannitol was found to accumulate in the chloroplasts, showed increased tolerance to oxidative stress in transgenic tobacco. As mannitol causes the removal of HO• and therefore increased mannitol is the indicator of enhanced stress protection [37]. From the present investigations, it is concluded that Cd metal stress negatively affects the physiology of *B. juncea* plants in terms of inhibition of mineral nutrient uptake and gaseous exchange parameters.

CONCLUSION

In response to the adverse effects of metal, compatible solutes and antioxidative defense system of the *Brassica juncea* plant got activated and proved beneficial to counteract the effects of metal stress by scavenging the ROS, generated during the stressed conditions.

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ABBREVIATION

ROS-reactive oxygen species, TCA-trichloro acetic acid, TPTZ-2,4,6-tripyridyl-S-triazine, GSH-reduced glutathione, DTNB-dinitrothiobenzoyl acid, IRGA-infrared gas analyzer, VPO-vapour pressure osmometer, Na⁺-sodium ions, K⁺-potassium ions.

CONFLICT OF INTERESTS

Declared none

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